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An extrachromosomal, inducible expression system for *Dictyostelium discoideum*

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ABSTRACT

Inducible expression systems are essential for the expression of toxic proteins and are very convenient for proteins that induce strong side effects such as retardation of growth or development. Currently available systems for use in *Dictyostelium* either do not have a very tight control over expression levels or use a combination of an integrating and an extra-chromosomal vector. We designed a new vector in which all components of the available 2-plasmid tetracycline-inducible system were combined onto a single extrachromosomal vector. Two types of inducible plasmids are presented, in which transcription is induced by adding or removing doxycycline, respectively. The location and orientation of the components was optimized in order to obtain a low background expression combined with high inducibility. The resulting vectors have a very low expression in the uninduced state (>1000-fold lower expression compared to that resulting from the act15 promoter), show a 10,000-fold induction of gene expression in a doxycycline concentration-dependent manner and are comparatively small (8.5 kb). With these new vectors, inducible gene expression is as easy as constitutive gene expression.

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1. Introduction

Ectopical gene expression is an indispensable tool for the analysis of gene function. High concentrations of gene product can be toxic to cells, which necessitates control over the level of gene expression. Several inducible expression systems have been developed for this purpose. In *Dictyostelium*, inducible transcriptional regulation has been described using a folate-dependent promoter element (Blusch et al., 1992) and using a DNA-damage induced promoter (Gaudet et al., 2001). A translational control system has also been developed, where a suppressor tRNA gene allows translation through a UAG stop codon (Dingermann et al., 1992; Pang et al., 2001). The system that is currently most widely used makes use of a recombinant transcription factor that activates transcription in a tetracycline-dependent manner (Gossen and Bujard, 1992). The reason

for its popularity is the high specificity and affinity by which the transactivator binds to the inducible promoter, resulting in a tight control of gene transcription. The levels of basal and induced expression of cells that are transfected with a tetracycline-inducible vector differs from clone to clone and must be determined individually. In the most favorable clones, expression of a reporter gene can be induced over a 100,000-fold range (Gossen and Bujard, 1992). The tetracycline-responsive system was originally developed for mammalian cells, but has been adapted for use in *Dictyostelium* (Blaauw et al., 2000). In this system, the transactivator is first integrated into the genome. Obtained stable cell lines are then transfected with an extra-chromosomal vector containing the controlled gene of interest. As in the mammalian system, the inducibility of gene transcription depends on the site and copy number of the integrated transactivator and expression levels need to be determined individually for each clone.

We have described a new set of modular expression vectors where transcription of the gene of interest is under the control of the *act15* promoter (Veltman et al. this is-

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sue), which results in high levels of transcription in both vegetative and developed cells. In this paper, we put all components of the existing 2-plasmid tetracycline-inducible system onto a single, Ddp1-based extrachromosomal vector. Despite the insertion of the transactivator gene, the size of the inducible vectors is still smaller than that of other Ddp1-based vectors such as pLittle and pJK1, that are used for constitutive expression (Levi et al., 2000; Pitt et al., 1992). Most importantly, since all components reside on one extrachromosomal vector, highly reproducible results are obtained from independent transfections without the need for clonal selection.

2. Materials and methods

2.1. Culture conditions and transformation of *Dictyostelium* cells

Dictyostelium AX3 cells were used for all experiments. Cells were cultivated on 9 cm Petri dishes or 6-wells plates containing HL5 medium. To induce or repress expression, doxycycline was added to the medium at 10 µg/ml. It should be noted that the rtTA-M2 and S2 transactivators that are used for the dox-on system can only be activated by doxycycline, which is a derivative of tetracycline. Addition of tetracycline has no effect. The transactivator tTA for the dox-off system (previously referred to as the tet-off system) is equally sensitive to tetracycline and doxycycline. For practical simplicity we therefore use doxycycline in all experiments. For transfection, 15 µl miniprep DNA (approximately 2 µg) was electroporated as described in (Howard et al., 1988). After 5–18 h G418 was added to the electroporated cells at a final concentration of 10 µg/ml.

2.2. DNA cloning

All vectors were constructed using standard cloning methods (Sambrook et al., 1989). Restriction enzymes were obtained from Fermentas, New England Biolabs and Roche. PCR amplifications were done using Phusion DNA

polymerase (Finnzymes). Open reading frames of PCR products were confirmed to be free of errors using DNA sequencing. Many vectors were created for testing purposes in order to determine which topology gave the lowest basal expression and the highest induced expression. Only the procedures for creating the final vectors listed in Table 1 will be described here.

The resistance cassette was created in exactly the same way as described (Veltman et al. this issue), except that the BamHI and XhoI site that are included in the primers for the amplification of the *act6* promoter and *mhcA* terminator, respectively, were swapped. As a result, the orientation of the resistance cassette in the inducible vectors is reversed compared to the vectors for constitutive expression. The inducible promoter was created as follows: Plasmid MB38 (Blaauw et al., 2000) that contains the inducible promoter TRE-P_{min} was digested with SmaI and EcoRI. A double stranded linker containing a HindIII site was created by annealing oligo 5'-aat tca agc ttc c-3' and oligo 5'-gga agc ttg-3'. This linker was ligated into the digested vector. The resulting vector contains the inducible promoter TRE-P_{min} in between a HindIII site and a BglII site.

The NgoMIV-flanked transactivator cassettes were created as follows: All transactivators share the same N-terminal and C-terminal region. The same primer pair 5'-gcggatccaaaatgtcaagattagat-3' and 5'-gctctagattaaccaccgtactcg-3' could thus be used for all amplifications. Plasmid pTet-off (Clontech), MB38 (Blaauw et al., 2000) and pUhrT62-1 (Urlinger et al., 2000) were used as PCR templates to obtain transactivator tTA, tTA_s* and rtTA-M2, respectively. PCR products were ligated in pBluescript II SK(-). Transactivator rtTA-M2 was converted to rtTA-M2_s* by replacing the BclI/BamHI fragment of rtTA-M2 with the BclI/BamHI fragment of tTA_s* (the BamHI site is that of pBluescript II SK(-) and is located downstream of the stop codon of the transactivator). Transactivator genes were subsequently excised with BamHI/XbaI and ligated in pDM202 digested with BglII/SpeI. Plasmid pDM202 is identical to plasmid pDM344 (Veltman et al. this issue) except that the multiple cloning site contains an additional NotI and MluI site.

Table 1

List of vectors that have been submitted to the *Dictyostelium* Stock Center. A minimal vector set from which, in combination with modules described (Veltman et al. this issue), all other vectors can be constructed is listed in bold. Annotated sequences of this minimal set have been submitted to GenBank.

Plasmid name	Transactivator	N-terminal tag	C-terminal tag	MCS	Resistance	GenBank Accession. No.
pDM309	tTA			BglII/SpeI	G418	EU908848
pDM310	rtTA-M2 _s *			BglII/SpeI	G418	EU908845
pDM359	rtTA-M2 _s *			BglII/SpeI	Hygromycin	EU908846
pDM360	rtTA-M2 _s *			BglII/SpeI	Blasticidin	EU908847
pDM331	rtTA-M2 _s *	GST		BglII/SpeI	G418	
pDM332	rtTA-M2 _s *	TAP		BglII/SpeI	G418	
pDM334	rtTA-M2 _s *	GFP		BglII/SpeI	G418	
pDM335	rtTA-M2 _s *	mRFPmars		BglII/SpeI	G418	
pDM338	rtTA-M2 _s *		TAP	BglII/SpeI	G418	
pDM340	rtTA-M2 _s *		GFP	BglII/SpeI	G418	
pDM341	rtTA-M2 _s *		mRFPmars	BglII/SpeI	G418	
pDM369	rtTA-M2 _s *	GFP		BglII/SpeI	Hygromycin	
pDM370	rtTA-M2 _s *		GFP	BglII/SpeI	Hygromycin	
pDM371	rtTA-M2 _s *	GFP		Gateway	Hygromycin	
pDM372	rtTA-M2 _s *		GFP	Gateway	Hygromycin	
pDM373	rtTA-M2 _s *			Gateway	Hygromycin	

2.3. Luciferase activity assay

Expression of luciferase was induced either by adding doxycycline to the desired concentration for dox-on systems, or by washing three times to remove the doxycycline from the medium for dox-off systems. After three days, cells were rinsed from the Petri dish and approximately 5×10^5 cells were transferred to an Eppendorf tube. Cells were spun down and lysed in 50 μ l reporter lysis buffer (Promega) according to manufacturers instructions. Luciferase activity of 5 μ l cell lysate was measured using the steadylite HTS assay (Perkin Elmer) and a Packard Lumi-Count luminometer. Protein concentration of the cell lysate was measured by a Bradford assay. Cells expressing luciferase under the control of an *act15* promoter were used as a reference for 100% activity.

3. Results and discussion

We constructed a set of extrachromosomal expression vectors for *Dictyostelium*, where expression of a gene of interest is under the control of a doxycycline-dependent transcriptional activator. The aim was to keep the vector as small as possible, with minimal expression in the uninduced state and a high level of expression in the induced state.

3.1. Minimizing basal expression

The small *Dictyostelium* expression vector pDM304 (Veltman et al. this issue) was used as starting material for the construction of the inducible expression vector. The constitutively active *act15* promoter that drives gene expression on pDM304 was first replaced by the inducible promoter TRE- P_{min} , which consists of 7 repeats of the TetO operator fused to a minimal fragment of the *act15* promoter (Fig. 1) (Blaauw et al., 2000). One of the greatest challenges when constructing inducible expression systems is to keep the expression levels in the absence of activated transactivator as low as possible. This unwanted expression can either stem from basal transcriptional activity of the inducible promoter itself, but may also come from sequences that are located upstream of the promoter. The TRE- P_{min} inducible promoter is only 430 bp long, whereas transcription activation can be regulated by sequences that are located much further upstream. To test a possible effect of upstream sequences, we first put the luciferase reporter gene under control of the inducible promoter TRE- P_{min} . Several different fragments were then placed upstream of the inducible promoter. Constructs were electroporated to *Dictyostelium* and luciferase activity of transfectants was measured. A plasmid expressing luciferase under control of the constitutively active *act15* promoter was used as a positive control and was normalized to represent 100% expression.

TRE- P_{min} was initially placed adjacent to the resistance marker gene, which is oriented in the opposite direction as the inducible expression cassette. Thus, the promoter of the resistance gene was upstream of the inducible promoter. Two *act6* promoter fragments of different lengths were tested to drive expression of the resistance gene. Both fragments resulted in robust resistance versus the selec-

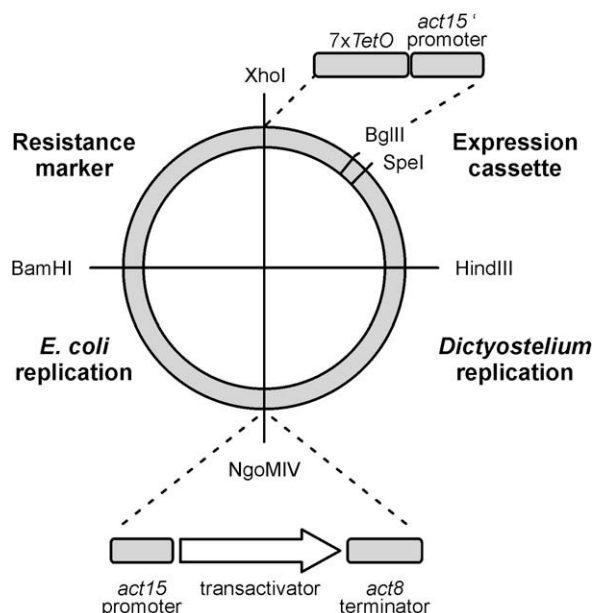


Fig. 1. Design of the inducible expression vector. The modular expression vector pDM304 was used as starting material for the construction of the inducible expression vector. The *act15* promoter that drives the expression of ectopic genes was replaced by the inducible promoter TRE- P_{min} . The transactivator cassette was inserted into the unique NgoMIV site.

tion marker, but when the longer *act6L*-P fragment (722 bp, identical to that used in (Nellen et al., 1984) and in many current *Dictyostelium* expression vectors) was placed upstream of TRE- P_{min} , a considerably higher expression of luciferase was measured when compared to the shorter *act6*-P fragment (222 bp) (see Fig. 2). Apparently, the longer fragment contains binding sites for transcription factors that initiate transcription in the opposite direction. Bidirectional transcription from *Dictyostelium* actin promoters has been reported previously (Knecht et al., 1993), suggesting that these may be a poor choice as a neighboring sequence for the inducible promoter.

We tried to reduce transcription activation by reversing the orientation of the resistance marker cassette, so that the terminator of the resistance gene becomes located upstream of the inducible promoter instead of the promoter of the resistance gene. Resistance cassettes with different terminator fragments were tested. Luciferase expression was relatively low with the *mhcA* terminator (<0.1% compared to *act15*-P::luc). An explanation for the 50-fold difference between the highest expression (*act8* terminator) and lowest expression (*mhcA* terminator) is not easily given. No transcription initiation is expected in the *Dictyostelium* genome near either the *act8* or *mhcA* terminator sequence and neither used terminator fragment is thus expected to contain transcription binding sites. However, the results confirm previous work where it was reported that terminator regions can influence transcription from downstream promoters (Maniak and Nellen, 1991).

In an attempt to reduce expression in absence of an activated transactivator even further, the orientation of the expression cassette was reversed, so that the inducible

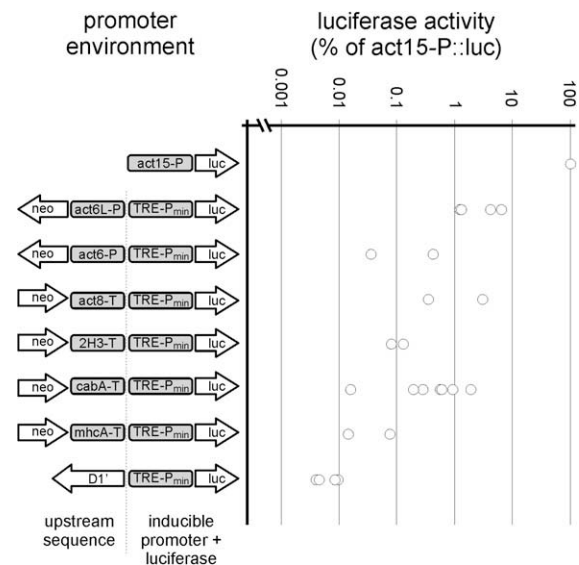


Fig. 2. Influence of upstream sequences on the basal transcriptional activity of the TRE-P_{min} promoter. Luciferase was put under the control of the TRE-P_{min} promoter. Several different fragments were then placed upstream of TRE-P_{min}. Constructs were transfected to AX3 cells and assayed for luciferase activity. A construct expressing luciferase under the control of the constitutive *act15* promoter was used as a positive control (100%). Each data point represents an independent single experiment performed on a different day.

promoter becomes located adjacent to the *Dictyostelium* replication cassette. At the border of this cassette lies the

truncated D1' gene that originates from *Dictyostelium* plasmid Ddp1 (Farrar et al., 1994). Being a remnant of an open reading frame, this region is not likely to contain any binding sites for transcription factors. Indeed, transcription activity from the inducible promoter was even lower in this case than with the *mhcA* terminator, potentially making this the best sequence to place adjacent to the inducible promoter.

3.2. Maximal induced expression

We proceeded to complete the 1-plasmid inducible expression vector by inserting a cassette that expresses the tetracycline-dependent transactivator tTA (Gossen and Bujard 1992) into the unique NgoMIV restriction site of the vector (Fig. 1). The resulting vector contains all the elements that are needed for inducible expression on one plasmid. Similar as the minimal expression in the absence of a transactivator, maximal induced expression may also depend on the environment of the inducible promoter. To screen for an optimal topology that yields both a low expression in the uninduced state and a high expression in the induced state, four vectors were constructed with different orientations of the expression cassette and the resistance marker cassette (Fig. 3A). The short *act6* promoter and the *mhcA* terminator fragment were chosen to drive the expression of the resistance marker as these gave lowest bidirectional transcription levels.

Co-expression of the transactivator leads to a small increase in luciferase activity, even in the presence of 10 µg/ml doxycycline which prevents the transactivator

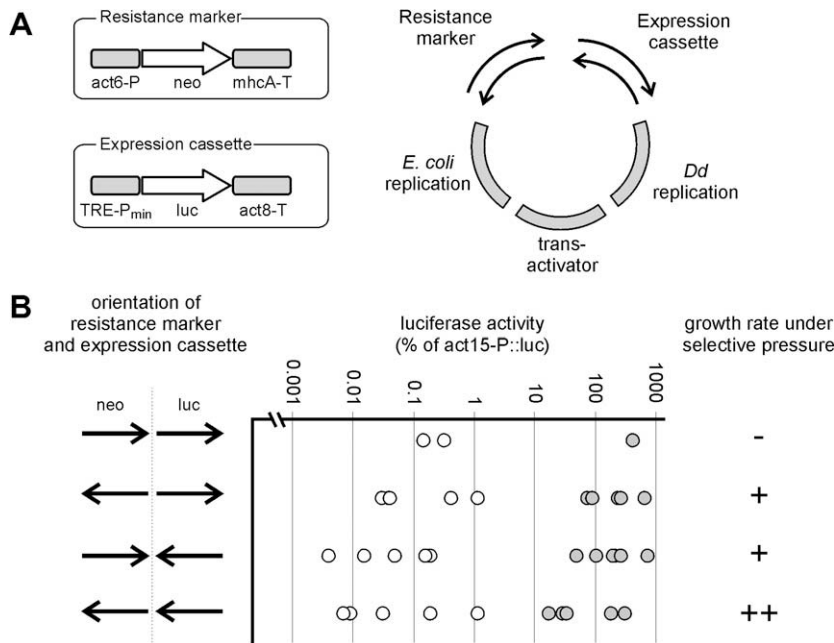


Fig. 3. Effect of vector topology on the expression range of the TRE-P_{min} promoter. (A) Expression vectors were created with all four possible orientations of the resistance marker cassette and expression cassette. The transactivator tTA was placed between the *E. coli* replication cassette and the *Dictyostelium* replication cassette. (B) Vectors were transfected to AX3 cells and luciferase activity was measured in the absence (white circles) or presence (grey circles) of 10 µg/ml doxycycline. Each data point represents an independent single experiment performed on a different day. Values are reported as a percentage of the positive control, which expressed luciferase under the control of the constitutive *act15* promoter (100%). Growth rate of transfected cells was scored semi quantitatively. Duplication in ~12 h (++) , 18 h (+) , 1 day (+/-) , 2 days (-) or longer than 3 days (---).

from binding to the tet operator (compare Fig. 2 and open symbols Fig. 3B). It should be noted here that doxycycline is a derivative of tetracycline and that the transactivator tTA is equally sensitive to both compounds. Uninduced expression is lowest when the inducible promoter is located adjacent to the partial D1' gene of the *Dictyostelium* replication cassette, consistent with the observations in the absence of a transactivator. Upon removal of doxycycline, the transactivator is allowed to bind the tet operator and to initiate transcription. As expected, this gave rise to a large increase in luciferase expression, often even surpassing the expression levels of luciferase under control of the strong, constitutively active *act15* promoter. This high induced expression level could be reached when either the *act6* promoter, *mhcA* terminator or D1' gene fragment was located upstream of the inducible promoter, indicating that the upstream environment of the inducible promoter does not greatly influence the maximal expression in the induced state. The greatest range between uninduced and induced expression (~10,000-fold) was obtained when the inducible promoter was located next to the partial D1' gene and with the resistance marker cassette in the opposite direction as the expression cassette.

The growth rate of *Dictyostelium* cells is an important selection criteria for the optimal inducible vector. When using the conventional 2-plasmid inducible expression system for *Dictyostelium*, a reduced growth rate is sometimes observed after integration of the transactivator into the genome (Blaauw et al., 2000). Similarly, when transfecting the 1-plasmid inducible expression vectors to *Dictyostelium*, a reduced growth rate was also noted for some vectors (Fig. 3B). This effect was stronger when the concentration of G418 was increased. In contrast, cells containing the parent vector pDM304 are robustly resistant to G418 over a wide concentration range. The growth rate of cells containing the inducible expression vector immediately returned to normal levels when the selective pressure was removed. These cells could still be induced to express luciferase, indicating that the vector was not lost from the population and the transactivator was still expressed. Combined observations imply that the transactivator itself is not cytotoxic, but increases the sensitivity of cells to G418. This effect may not be specific for *Dictyostelium* cells as similar results have been reported for tetracycline-inducible systems in human stem cells (Bryja et al., 2003). Fortunately, the cells carrying the plasmid with the greatest range between uninduced and induced expression showed a good growth rate and therefore this topology was used to construct new inducible expression vectors.

The screen for optimal inducible vectors was continued by testing the transcriptional activation generated by different transactivators. The transactivator consists of two fragments. The N-terminal fragment is the bacterial tetracycline-repressor TetR. This bacterial gene is fused on the C-terminus to a fragment of the strong transcriptional activator VP16 from the herpes simplex virus (Gossen and Bujard, 1992). Two different transactivators were originally reported that differed in the length of the VP16 fragment. Both transactivators had similar properties when expressed in human cells. The tetracycline-repressor fragment of the transactivator was later modified so that it

binds to the tet-operon in the presence of doxycycline instead of in its absence. Two different versions of this reversed repressor were reported, M2 and S2, which varied in their sensitivity to doxycycline. We fused each of the three tet-repressor fragments to each of the two VP16 fragments, resulting in six different transactivators. The transactivators were placed under the control of the *act15* promoter and *act8* terminator and ligated into the NgoMIV site of the inducible expression vector (Fig. 1). Expression of luciferase in the absence and presence of 10 μ M doxycycline, as well as the growth rate of transfected cells under selective pressure is shown in Fig. 4. Considerable variation in both growth rate and expression levels were observed. The best transactivator for a dox-off system was found to be tTA, the originally reported transactivator. Luciferase expression is highly inducible (10,000-fold) with this transactivator and cells maintaining this vector show good growth rate under selective pressure. For a dox-on vector, rtTA-M2_s* was found to be the best transactivator. Remarkably, the luciferase expression levels of cells containing a vector where luciferase transcription is controlled by rtTA-M2_s* is very similar compared to cells containing a vector where luciferase transcription is controlled by tTA; Expression of uninduced cells is about 0.01% when com-

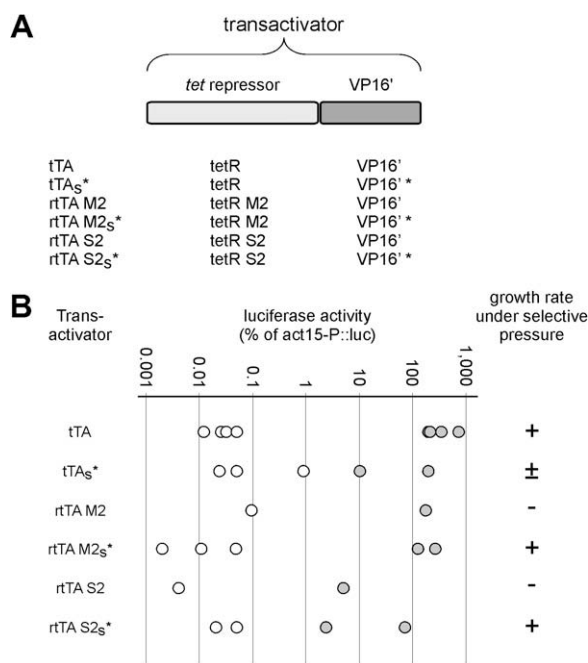


Fig. 4. Effect of transactivator composition on dynamic expression range. Three different tet-repressor fragments (Gossen and Bujard, 1992; Urlinger et al., 2000) were fused to two different VP16 fragments (Blaauw et al., 2000; Gossen and Bujard, 1992). The resulting transactivators were placed under control of an *act15* promoter and *act8* terminator and ligated into the inducible expression vector. Luciferase activity of transfected cells was measured in the uninduced (white circles) and induced (grey circles) state and is reported as a percentage of cells expressing luciferase under control of the *act15* promoter. Each data point represents an independent single experiment performed on a different day. Growth rate of transfected cells was scored semi quantitatively. Duplication in ~12 h (++) , 18 h (+) , 1 day (+/-) , 2 days (-) or longer than 3 days (---).

pared to cells containing a vector where transcription is under control of the *act15* promoter and when the rtTA-M2_s* transactivator is fully activated, the resulting expression of luciferase is similar to that of cells expressing luciferase under control of the *act15* promoter. Growth rate of transfected cells under selective pressure was only slightly reduced compared to that of wild type cells without selective pressure. It should be noted that the orientation of the transactivator cassette did not change the range of inducible expression in any experiment.

3.3. Properties of the inducible vector

The above screen resulted in two optimal vectors, pDM309 with tTA where transcription is repressed by adding doxycycline, and pDM310 with rtTA-M2_s* where transcription is activated by adding doxycycline. We performed a dose response experiment by adding different concentrations of doxycycline and measured the resulting levels of luciferase expression (Fig. 5A). The inflection points of both curves are at a concentration of approximately 0.1 µg/ml doxycycline, but the slope of the curve for the dox-off vector is steeper than that of the dox-on vector. The more shallow slope of the dox-on vector potentially allows exact fine-tuning to desired expression levels by titrating the doxycycline concentration.

The speed by which the levels of luciferase increase after the start of induction was measured for both the dox-on vector and the dox-off vector (Fig. 5b, solid markers). Induction of expression is easier for the dox-on system, where doxycycline is simply added to the desired concentration. A 30% increase in luciferase levels was observed 15 min after start of induction for the dox-on system; 100-fold induction was observed after about 2 h. To induce gene expression with the dox-off system, the doxycycline was removed by washing cells three times with a volume of approximately 50 times the total cell volume. Apparently, cells lost the doxycycline only relatively slowly after the washout, as luciferase expression levels did not increase until after 2 h. Similar kinetics of expression were observed with cells starving in buffer (Fig. 5b, open markers). Expression was induced by adding/removing doxycycline after 2 h of starvation. A 1000-fold increase was observed for the dox-on vector after 4 more hours of starvation, which is similar to the response of vegetative cells. For the dox-off system there again was a 2 h lag phase before luciferase levels started to increase.

In principle, the increase of expression levels upon induction can have two potential causes: (1) a small fraction of cells exhibits a high expression and this fraction increases when cells are stronger induced or (2) the expression in all cells increases evenly as cells are stronger induced. We examined the cell-to-cell variation of expression by using green fluorescent protein under control of the dox-on transactivator rtTA-M2_s*. Expression was either fully induced (10 µg/ml doxycycline), half induced (0.5 µg/ml doxycycline) or uninduced (no doxycycline). A vector with green fluorescent protein driven by the constitutively active *act15* promoter was used as a positive control. Confocal images of the cells are shown in Fig. 6. Image acquisition parameters were identical for each picture. Average

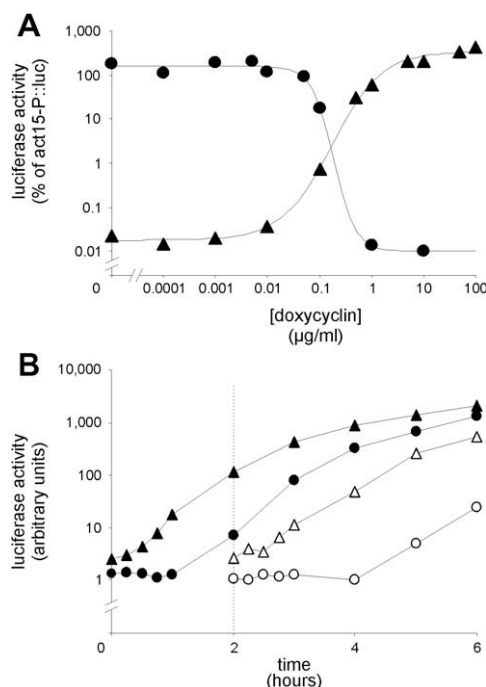


Fig. 5. Dose-response and kinetics of inducible expression. (A) Cells were transfected with a vector expressing luciferase under control of the rtTA-M2_s* transactivator (triangles), or the tTA transactivator (circles). Expression was induced by adding/removing doxycycline to the indicated levels and luminescence was measured after 3 days. (B) Unstimulated cells from (A) were split in two batches. Luciferase expression of one half was induced at $t = 0$ by adding doxycycline to 10 µg/ml (dox-on, solid triangles) or by removing doxycycline from the medium (dox-off, solid circles). Samples were taken at indicated time points and assayed for luciferase activity. The other half of the cells were starved by shaking them in phosphate buffer. At $t = 2$ h, luciferase expression was induced by either removing doxycycline from the medium (dox-off, open markers) or adding doxycycline to 10 µg/ml. Samples were taken at indicated time points and assayed for luciferase activity (open markers). The presented data is the average of two independent experiments.

fluorescence intensity of the cytosol of the cells was quantified using ImageJ. The range of fluorescence, from 0 to 255, was divided into six equal groups and the fraction the population in each group is plotted in Fig. 6B. It is immediately clear from the figure that the distribution of fluorescence is skewed, with a large fraction of cells with low expression and a small fraction of cells that have a relatively high expression. This distribution is similar for constitutive expression as for inducible expression. The figure also shows that when the doxycycline concentration is reduced so that half-maximal expression is induced, there are only few cells in the two fractions with the highest expression levels. The populations with average fluorescence are declined and as a result, there are more cells in the lowest fraction, with very low expression. On the basis of these results, we conclude that inducible expression does not alter the cell-to-cell distribution of expression.

3.4. Constructed vectors for use

A plasmid map of a basic expression vector with the dox-on transactivator rtTA-M2_s* (pDM310), is shown in

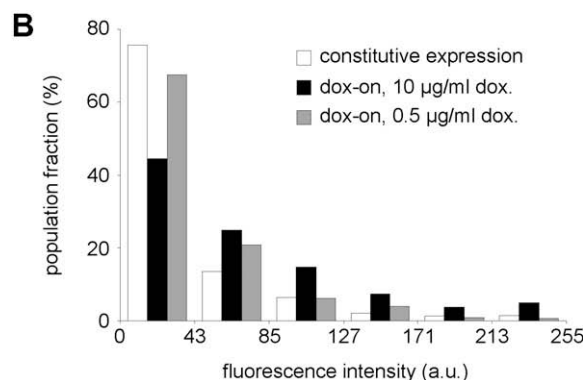
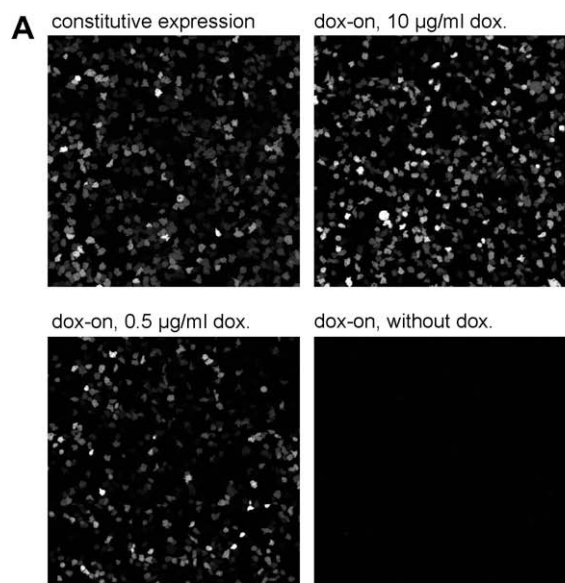


Fig. 6. Cell-to-cell distribution of expression. (A) vectors were constructed that expressed green fluorescent protein under control of either the *act15* promoter (constitutive expression) or the transactivator *rtTA-M2s** (dox-on). Expression of green fluorescent protein was maximally induced (10 µg/ml doxycycline), half-maximal (0.5 µg/ml doxycycline) or uninduced (without doxycycline). Confocal images of cells were taken using the same parameters for each strain. (B) Fluorescence of each cell in (A) was measured and yielded a value between 0 and 255. This range was divided into 6 equal groups and the percentage of cells in each group was plotted.

Fig. 7. The inducible expression vectors are derived from the modular vector pDM304 and share the same characteristics (Veltman et al. this issue). Thus, fusion tags and Gateway conversion cassettes that are available for the modular vectors can also be used for the inducible vectors. A large number of vectors can be created using the individual components. Some have already been constructed and have been deposited at the *Dictyostelium* stock center (www.dictybase.org) for distribution (Table 1). The advantages of the small 1-plasmid inducible system over the 2-plasmid system are obvious by the ease of experimental design. Cloning of the expressed gene, transfection, selection and characterization of the cells is now as easy with an inducible vector as with a vector for constitutive expression.

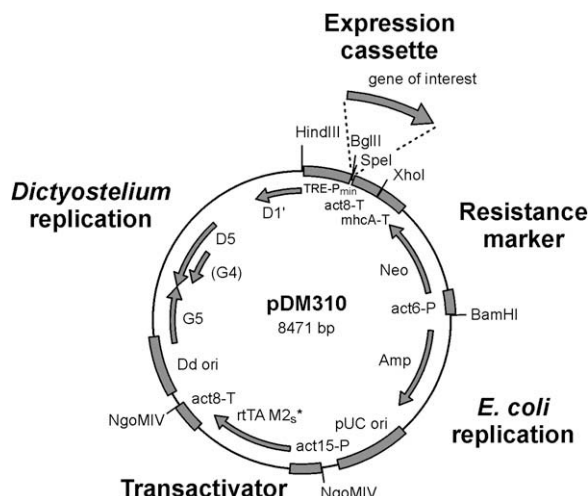


Fig. 7. Vector map of pDM410, an extrachromosomal, dox-on inducible expression vector with G418 as a selection marker. The restriction sites that border the five functional regions are indicated. A gene of interest can be cloned in between the *BglII* and *SpeI* site.

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